COMMENTARY

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To B-(RAF) or Not to Be

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The identification of targetable mutations has revolutionized the therapy of metastatic melanoma. In particular, BRAF and MEK inhibitors have a well-documented impact on overall survival in metastatic disease. However, therapeutic success is highly dependent on the correct identification of these mutations. We discuss the impact of molecular heterogeneity in this context.

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Melanoma is frequently driven by activating mutations in the MAPK kinase pathway (Thomas *et al.*, 2007), with BRAF and NRAS being the most common. In order to target the pathway when activated, several small molecule inhibitors have been developed, including the BRAF inhibitors vemurafenib, dabrafenib, and LGX 818, and the MEK inhibitors trametinib, selumetinib, Mek163, and cobimetinib. These molecules can be used successfully in metastatic melanoma, and may have significant impact on survival (Chapman *et al.*, 2011; Flaherty *et al.*, 2012; Kirkwood *et al.*, 2012; Ascierto *et al.*, 2013; Robert *et al.*, 2013). Unfortunately, despite impressive tumor regression initially, the tumors in most patients ultimately develop resistance (Dummer and Flaherty, 2012). These treatments are ordinarily initiated on the basis of one BRAF mutation found in one tumor lesion removed from a patient. Saint-Jean *et al.* (2014) now demonstrate that this approach may fail to identify all patients who may profit from this sort of targeted approach.

Tumors are heterogeneous mixtures of cells with diverse genetic, epigenetic, and phenotypic alterations that show impressive transcriptional plasticity (Hoek *et al.*, 2008). Although microenvironmental cues such as hypoxia (Widmer *et al.*, 2013) or tumor–stromal interactions may modulate cellular behavior, fixed-genetic heterogeneity is also likely to have a large role in tumor progression and responses to therapy.

Melanoma: a heterogeneous disease in many ways

We used to think of a malignant tumor as a uniform clonal cell accumulation, with a stable transcriptome that facilitated high proliferation, no apoptosis, and resistance to immune defense mechanisms. Today, we understand a metastasizing malignancy as a mixed organ–like population that is heterogeneous and transcriptionally plastic, and supported by a plethora of benign reprogrammed bystander cells, such as fibroblasts and macrophages, and other immune and endothelium cells that form a tumor stroma. Their incredible flexibility to adapt to various niches is made possible by unrestricted access to developmental programs that allow for dedifferentiation, proliferation, and migration. The pathogenic (re)activation of these embryonic programs may be modulated by a wide variety of epigenetic mechanisms as well as by genetic and environmental factors.

Melanomas are neural crest–derived neoplasms, and, as such, tumor cells within each metastasis may express neural crest markers such as CD271 and Sox10, and may also exhibit features of different neural crest derivatives such as mesenchymal, neural, and smooth muscle cells (Civenni *et al.*, 2011). This heterogeneity seems to be shaped by immune mechanisms, as has been shown by experimental xenografting of melanoma cells in nude, NOD/SCID, or highly immunocompromised NSG mice. In this way, the presence of natural killer cells was found to affect the resulting heterogeneity of the xenografts. Thus, the cellular composition of xenografts derived from CD271-positive cells only reflected the corresponding parental tumors in the presence of some immune functions (Civenni *et al.*, 2011).

Abrupt interruption of growth-promoting signaling pathways such as the MAPK pathway, spontaneous inflammation or inflammatory reactions established by immunotherapy, and hypoxia may cause stress reactions within a tumor that lead to transcriptional adaptations, including reduced melanocytic differentiation. This phenotypic switch may be interpreted as the equivalent of epithelial mesenchymal transition (EMT) or the induction of stemness, and it includes features of senescence (Braumuller *et al.*, 2013). This adaptive phenotype switch (Hoek *et al.*, 2008) is a potent treatment-resistant mechanism, with relevance to all currently available cancer therapies, including chemotherapy, irradiation, targeted therapy, and, as recently documented, immunotherapy (Landsberg *et al.*, 2012), because it effects immunogenicity through epigenetic alterations and rewires signaling networks (Holzel *et al.*, 2013).

In this context, activating mutations affecting the MAPK pathway appears to be an essential condition for all tumor cells. The paper of Saint-Jean *et al.* (2014) convincingly demonstrates that even for a major driver mutation such as BRAF, there is no homogenous distribution in an individual who has metastatic disease. Indeed, there are several reports that provide evidence for the relapse of BRAF wild-type tumor cell populations after initially successful targeted therapy. A comprehensive analysis of melanoma biopsies collected before, during vemurafenib, and at relapse demonstrated reactivation of the MAPK kinase pathway, as observed by elevated ERK1/2 phosphorylation levels at relapse associated with secondary *NRASQ61* mutations or *MEK1Q56P* or *MEK1E203K* mutations (Trunzer *et al.*, 2013). It is reasonable to assume that

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Clinical Implications

- Heterogeneity on genetic, epigenetic, and phenotypic levels is a key feature of melanoma.
- Genetic heterogeneity may include driver mutations such as BRAF or NRAS within individual patients.
- Genetic investigations of single lesions may not always accurately capture the genetic state of disease in a single individual.

these mutations were already present before vemurafenib therapy, which established a selective pressure favoring tumor cell clones with these genetic alterations.

The paper of Saint-Jean *et al.* (2014) reported that a discordance in BRAF mutation results in two different metastasis in a single individual in 13% of the patients. Two patients with discordant mutation test results were treated with the BRAF inhibitor vemurafenib. Both of these patients appeared to ''profit'' from that therapy, one with stable disease and the other with partial remission. A possible explanation is that most tumor cells contain the mutation and therefore can be targeted. However, there is also a possibility that the effects of the targeted therapy depend partly on off-target effects such as modulation of the tumor microenvironment and releasing preexisting immune responses.

These data (Saint-Jean *et al.*, 2014) have been carefully generated, and they challenge our current standard of care. Certainly, technical problems during BRAF testing have been excluded as well as sampling errors. Finally, it is reasonable to assume that these findings reflect one aspect of the heterogeneous genetic background of the malignancy. In addition, the data raise a question about the need to examine testing of several tumor lesions before excluding a patient from a targeted therapy. New, more sensitive sequencing techniques will allow us to quantify the frequency of a given genetic alteration within one tumor lesion as well as in circulating tumor cells in the blood. In our opinion, this information will soon be clinically relevant. Targeted therapy can work impressively, but there is plenty of room for optimization. Today, we play the piano with a single finger, but with better knowledge of the pathways and underlying heterogeneity that drive tumor progression, we are well on our way to using full hands.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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